Genetic dissection of plant cell-wall biosynthesis

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Abstract

The plant cell wall is a complex structure consisting of a variety of polymers including cellulose, xyloglucan, xylan and polygalacturonan. Biochemical and genetic analysis has made it possible to clone genes encoding cellulose synthases (*CesA*). A comparison of the predicted protein sequences in the *Arabidopsis* genome indicates that 30 divergent genes with similarity to *CesAs* exist. It is possible that these cellulose synthase-like (Csl) proteins do not contribute to cellulose synthesis, but rather to the synthesis of other wall polymers. A major challenge is, therefore, to assign biological function to these genes. In an effort to address this issue we have systematically identified T-DNA or transposon insertions in 17 *Arabidopsis* Csls. Phenotypic characterization of 'knock-out' mutants includes the determination of spectroscopic profile differences in mutant cell walls from wild-type plants by Fourier-transform IR microscopy. A more precise characterization includes cell wall fractionation followed by neutral sugar composition analysis by anionic exchange chromatography.

Introduction

The cell wall is a structurally complex constituent of a plant cell. It is a flexible and active metabolic cell compartment that is remodelled during growth and development and in response to changes in environmental conditions. Despite its importance, there is a scarcity of information with respect to the enzymes responsible for the synthesis of the array of polysaccharides that make up the bulk of plant cell walls. The isolation of mutations in the *Arabidopsis root swelling 1* (*RSW1*)/*CesA1 gene* [1,2], which encodes a subunit of a putative cellulose synthase complex, provided the first functional link between cellulose biosynthesis and a genetic locus.

Key words: *Arabidopsis*, cellulose synthase, Fourier-transform IR, polysaccharide biosynthesis.

Abbreviations used: Ces, cellulose synthase; Csl protein, cellulose synthase-like protein; RSW, root swelling 1.

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Computer-based comparisons of RSW1/CesA protein sequences with predicted protein sequences in the *Arabidopsis* genome have revealed the existence of a large family of at least 40 genes that can be grouped into either cellulose synthase (*CesA*) or structurally related 'cellulose synthase-like' (*Csl*) sequences (*CslA*, *CslB*, *CslC*, *CslD*, *CslE* and *CslG*) [3,4]. The cellulose synthase-like genes range in their similarity to the *CesA* family from approx. 7% to 35% identity at the amino acid level. Regardless of the degree of sequence similarity however, members of the *CesA*/*Csl* gene family have features that have been proposed to be the hallmarks of processive glycosyltransferases [5,6] and appear to belong to family 2 of the inverting nucleotide-diphosphate-sugar glycosyltransferases that synthesize repeating β-glycosyl unit structures [7]. For example, all predicted CesA and Csl protein sequences contain the conserved Asp-Asp-Asp-Gln-Xaa-Arg-Trp motif that is thought to define the sugar-binding and catalytic sites of these enzymes. In addition, on the basis of hydrophobicity prediction all CesA/Csl members appear to be integral membrane proteins with three to six transmembrane domains in the C-terminal region of the protein and one or two transmembrane domains at the N-terminus.

An account for the diversity of cellulose synthase homologues could be attributed to cell-type specificity in cellulose biosynthesis and/or in the formation of other cell-wall polymers such as xyloglucans or mixed linked glucans. Indeed, the expression levels of *CesA* and *Csl* families based on expressed sequence tag representation in GenBank indicates that there is a broad range of expression from as high as 54% for the *CesA*s to as low as 2% for the *CslB*s [8], suggesting that some of the Csls may catalyse the synthesis of only minor cell-wall components or that they might exhibit cell- or tissue-type specificity.

Genetic analysis involving the isolation of mutations that disrupt cell-wall biosynthesis has been exploited on only a limited basis [9]. Mutations that have been isolated so far have been restricted to those affecting either polysaccharide backbone modification [10] or cellulose synthesis [9]. The lack of mutations isolated in genes

affecting non-cellulosic polysaccharide backbones could be credited to genetic redundancy or subtlety of phenotype. If Csls are processive glycosyltransferases responsible for the synthesis of major wall polysaccharides, the fact that some are present in the genome in tandem arrays and have overlapping expression patterns suggest that they share redundant functions [8].

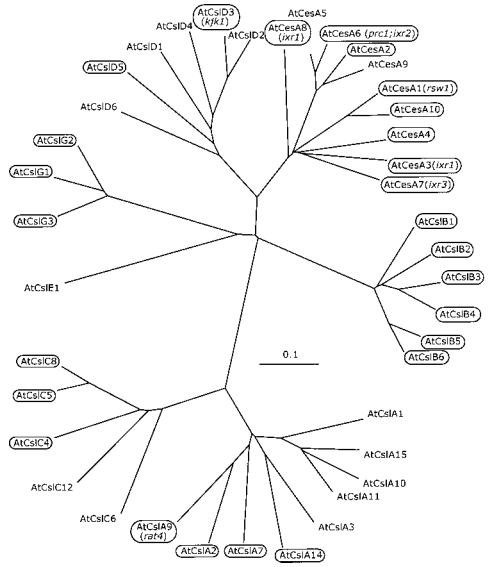
At present, only two mutations in *Csl* genes exist with an easily discernible phenotype. The *kojak* (*kjk*) mutation, which is a loss-of-function mutation in *CslD3*, causes root-hair initials to have weakened cell walls that burst, resulting in roots with no root-hairs [11]. Despite *CslD3* gene expression throughout the plant, the lack of any obvious phenotype in other tissues implies that it might be too subtle to be appreciated, based on morphology alone. Another *Csl* mutation is a dominant T-DNA insertion in the *CslA9* gene (*rat4*), which confers resistance to *Agrobacterium* root transformation [12,13]. These two examples illustrate the difficulty in predicting phenotypes that might result from *Csl* loss-of-function mutations. In addition, in neither case is it clear what cell wall polymer is affected or missing in these mutants.

The outstanding challenge remains in determining *Csl* function in *Arabidopsis*. It should be possible to gain insight into this problem by utilizing a number of reverse genetic tools including large T-DNA and transposon collections [14,15], and interference RNA methods [16]. One of our primary efforts, therefore, has been to isolate disruptions in individual *Csl* genes by screening T-DNA- or transposon-mutated lines. Figure 1 illustrates our progress. Phenotypic characterization of these mutant lines has involved IR spectromicroscopy as a first assay to determine gross defects in wall polymer structure/composition.

Figure 1

Mutations in *Arabidopsis CesA*/*Csl* genes

An unrooted, bootstrapped tree showing *Arabidopsis CesA* and *Csl* genes grouped into six major families. Genes that are boxed indicate genes where a T-DNA insertion, a transposon, or point mutation has been isolated. This tree is courtesy of Todd Richmond.



Materials and methods

The Advanced Light Source at the Lawrence Berkeley National Laboratory is a synchrotron light source operated as a National User Facility by the US Department of Energy. Beamline 1.4.3 at the Advanced Light Source [17] is dedicated to IR spectromicroscopy, and collimated synchrotron light serves as an external input to a Thermo Nicolet Instruments (Madison, WI, U.S.A.) Magna 760 Fourier-transform IR spectrometer. The modulated light is then passed through a Nic-Plan IR microscope to perform either transmission or reflection microscopy. The sample stage of the microscope is controlled in the x–y plane, allowing automated spectral measurements across samples with steps that can be 1 μm wide. Common glass optical components (other than the visual eyepieces and a $\times 10$ sample-inspection objective) are not found in the Nic-Plan IR microscope, which instead relies on all-reflecting Schwartzchild optics. A $\times 15$ objective was used for all experiments. To minimize IR absorption by CO_2 and water vapour in ambient air, the optics were purged using dry air. Spectra for these experiments were collected in single-beam reflection mode with a wavelength resolution of 2 cm^{-1}, Happ-Genzel apodization and 1028 scans co-added for Fourier transform processing to produce one spectrum. In reflectance mode, the upper Cassegrainian objective serves a dual role as both the objective and condenser. Each resulting single-beam spectrum from leaf samples was normalized to a gold reference spectrum recorded under the same conditions. This normalization procedure removes the source – beamsplitter–detector efficiency functions, and minimizes IR spectral contributions from intervening water vapour and CO_2. Absorbance spectra were calculated subsequently. The lower energy limit for spectra acquired through the IR microscope is 600 cm^{-1}, the cut-off of its liquid N_2-cooled MCT (mercury-cadmium-

telluride) type-A detector. All spectra presented here were acquired over the region of 4000–650 cm^{-1} (2.5–16 μm). The samples themselves were 1 cm^2 leaf discs cleared in chloroform/methanol [1:1 (v/v)]. All solvents were of ultrapure grade. Samples were cleared with several changes of solvent until no additional pigment was detected in the discarded liquid. The cleared discs were air-dried and stored on porcelain spot plates until analysis.

Results and discussion

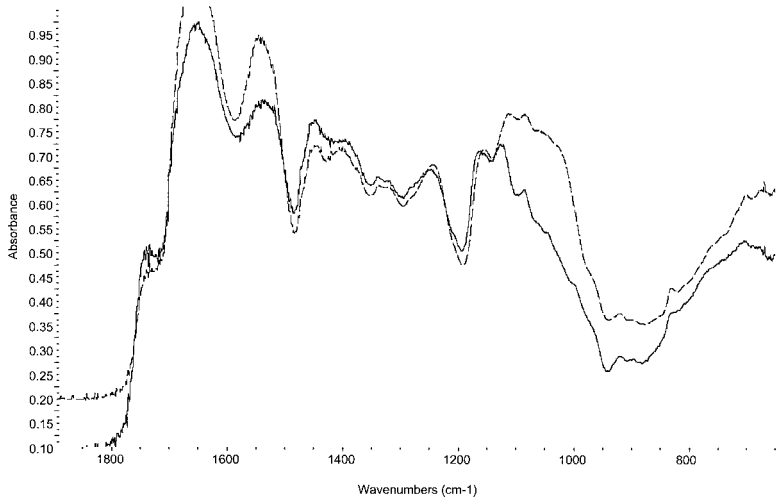
Mid-IR spectroscopy (2.5–16 μm wavelength; 4000–650 cm^{-1}) measures the contribution from vibrations of particular organic and inorganic functional groups within molecules. IR methods have a very long history of contribution to analytical chemistry. Unlike many types of microscopy, IR spectromicroscopy provides chemical analytical information on the composition of plant tissues, and acts as an *in situ* contrast reagent for biological tissues. The earliest research on IR microscopes dates back to about 1985. A drawback of the old optical systems for IR microscopes was the resulting diffraction effects if the aperture was decreased to limit the field of view to a small region of interest. At the same time, less light overall reaches the detector, and hence the signal-to-noise ratio decreases. In the earliest applications of this methodology to plant science [18], spectra were collected at lower resolution (8 cm^{-1} data spacing) to reduce time of acquisition. In a plant cellular context where we wish to study neighbouring cell types, we would ideally like to be able to collect spectral data at the diffraction limit, or a few microns in each spatial dimension. Only synchrotrons and free electron lasers can achieve this [19,20]. The brightness of conventional benchtop IR sources is simply too low by 2–3 orders of magnitude.

Based on the advantages offered by IR spectromicroscopy,we have employed this technique to provide a 'snapshot' of the cell-wall composition in mutant lines that we have identified. By way of example, a comparison of the mid-IR spectra from leaf discs of *Arabidopsis* wild-type Columbia versus a strain with a T-DNA insertion in the *CslB6* gene is provided (Figure 2). Principally, the absorbance changes in the polysaccharide 'fingerprint' region of 1200–900 cm^{-1} demonstrated an increased shoulder in the cellulosic region of the spectrum from 950 to 1065 cm^{-1} [21]. Assignment of peaks in this region is difficult, since the absorptions may arise from complex deformations of the molecule. They may be characteristic of molecular symmetry, or combination bands arising from multiple bonds deforming simultaneously. Based on normal-mode calculations for purified extracts of crystalline cellulose [22], very strong absorptions at 1060 and 1035 cm^{-1} can definitely be attributed to pyranose ring C—O–C bonds. However, complicating interpretations in this region are strong resonances from uronic-acid-rich pectins at 1011 and 1038 cm^{-1}. To decide which of these two cell-wall components is affected by the B6 lesion, we will

Figure 2

Differences between wild-type and *CslB6* mutant leaf IR absorbance spectra

Wild-type is represented by the solid line and the *CslB6* mutant by the broken line. Each plot represents an average of five or six samples for each genotype. Refer to main text for details.



use standard chemical fractionation schemes [23] to correlate with the mid-IR spectra. At this point, we see that IR has a useful heuristic role in selecting the most relevant chemical fractions to prepare from plant tissue.

References

1 Pear, J. R., Kawagoe, Y., Schreckengost, W. E., Delmer, D. P. and Stalker, D. M. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 12637–12642
2 Arioli, T., Peng, L., Betzner, A. S., Burn, J., Wittke, W., Herth, W., Camilleri, C., Hofte, H., Plazinski, J., Birch, R. et al. (1998) Science **279**, 717–720
3 Cutler, S. and Somerville, C. (1997) Curr. Biol. **7**, R108–R111
4 Richmond, T. A. and Somerville, C. (2000) Plant Physiol. **124**, 495–498
5 Saxena, I. M., Brown, Jr, R. M., Fevre, M., Geremia, R. A. and Henrissat, B. (1995) J. Bacteriol. **177**, 1419–1424
6 Henrissat, B., Coutinho, P. M. and Davies, G. J. (2001) Plant Mol. Biol. **47**, 55–72
7 Campbel, J. A., Davies, G. J., Bulone, V. and Henrissat, B. (1997) Biochem. J. **326**, 929–939
8 Richmond, T. A. and Somerville, C. R. (2001) Plant Mol. Biol. **47**, 131–143
9 Fagard, M., Hofte, H. and Vernhettes, S. (2000) Plant Physiol. Biochem. **38**, 15–25
10 Reiter, W.-D. and Vanzin, G. (2001) Plant Mol. Biol. **47**, 95–113
11 Favery, B., Ryan, E., Foreman, J., Linstead, P., Boudonck, K., Steer, M., Shaw, P. and Dolan, L. (2001) Genes Dev. **15**, 79–89
12 Nam, J., Mysore, K. S., Zheng, C., Knue, M. K., Matthysse, A. C. and Gelvin, S. B. (1999) Mol. Gen. Genet **261**, 429–438
13 Gelvin, S. B. (2000) Annu. Rev. Plant Physiol. Plant Mol. Biol. **51**, 223–256
14 McKinney, E. C., Ali, N., Traut, A., Feldmann, K. A., Belostotsky, D. A., McDowell, J. M. and Meagher, R. B. (1995) Plant J. **8**, 613–622
15 Krysan, P. J., Young, J. C., Tax, F. and Sussman, M. R. (1996) Proc. Natl. Acad. Sci. U.S.A. **93**, 8145–8150
16 Chuang, C. F. and Meyerowitz, E. M. (2000) Proc. Natl. Acad. Sci. U.S.A. **97**, 4985–4990
17 Martin, M. C. and McKinney, W. R. (1998) Proc. Mater. Res. Soc. **524**, 11–20
18 McCann, M. C., Hammouri, M., Wilson, R., Belton, P. and Roberts, K. (1992) Plant Physiol. **100**, 1940–1947
19 Jamin, N., Dumas, P., Moncuitt, J., Fridman, W. H., Teillaud, J. L., Carr, G. L. and Williams, G. P. (1998) Proc. Natl. Acad. Sci. U.S.A. **95**, 4837–4840
20 Raab, T. K. and Martin, M. C. (2001) Planta **213**, 881–887
21 Kataoka, Y. and Kondo, T. (1998) Macromolecules **31**, 760–764
22 Cael, J. J., Gardner, K. H., Koenig, J. L. and Blackwell, J. (1975) J. Chem. Phys **62**, 1145–1153
23 Peng, L., Hocart, C. H., Redmond, J. W. and Williamson, R. E. (2000) Planta **211**, 406–414

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A multigene family of glycosyltransferases in a model plant, *Arabidopsis thaliana*

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Abstract

Glycosyltransferases transfer sugars from NDP-sugar donors to acceptors. The multigene family of transferases described in this paper typically transfer glucose from UDP-glucose to low-molecular-mass acceptors in the cytosol of plant cells. There are 107 sequences in the genome of *Arabidopsis thaliana* that contain a consensus, suggesting they belong to this Group 1 multigene family. The family has been analysed phylogenetically, and a functional genomics approach has been applied to explore the relatedness of sequence similarity to catalytic specificity and stereoselectivity. Enzymes belonging to this class of transferases glycosylate a vast array of acceptors, including natural products such as secondary metabolites and hormones, as well as xenobiotics absorbed by the plant, such as herbicides and pesticides. Conjugation to glucose potentially changes the activity of the acceptor molecule and invariably changes its location within the plant cell. Using the genomics approach described, a platform of knowledge has been constructed that will enable an understanding to be gained on the role of these enzymes in cellular homoeostasis, as well as their activity in biotransformations *in vitro* that require strict regioselectivity of glycosylation.

Key words: catalysis, genomics, glycosyltransferase, natural product chemistry, plants.

Abbreviation used: IAA, indole-3-acetic acid.

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Introduction

The completion of genome sequencing programmes has opened up new opportunities for studying the relationship of sequence and struc-